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ACCUMULATION OF DEBRISOQUIN-^{14}C BY THE HUMAN PLATELET

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Abstract—Debrisoquin (3,4 dihydro-2 (1H)-isoquinoline carboxamidine) is accumulated in human platelets against a concentration gradient. The transport mechanism for this drug is energy dependent and saturable. Various amines and drugs acting on the adrenergic neuron, which are also accumulated by the platelet, compete with debrisoquin for a common transport system. Serotonin and debrisoquin demonstrate an equal affinity for the transport system, while tyramine, bretylium and guanethidine have a lesser affinity. Ouabain produces a mixed type of inhibition but reserpine had no effect. The uptake mechanism for organic bases in the human platelet is relatively nonspecific, whereas the intracellular binding site(s) for these compounds may have greater specificity.

DEBRISOQUIN (3,4 dihydro-2 (1H)-isoquinoline carboxamidine) is a drug which acts on the adrenergic neuron, has hypotensive properties in animals[1] and man,[2] and also has pharmacologic effects on the human platelet.[3] Recent studies of the influence of debrisoquin on this cell, *in vivo* and *in vitro*, indicate that this drug competitively inhibits the uptake of serotonin by the platelet, releases this amine from the cell and noncompetitively inhibits platelet monoamine oxidase activity.[3]

In this study, the uptake of debrisoquin-^{14}C by the human platelet has been characterized and the effects of various drugs on the accumulation and storage of this compound were investigated.

METHODS

Platelets were isolated from healthy human subjects as previously described[4] and were washed twice in Tris-buffered saline (pH 7·5) containing 1·3 mM EDTA. Cells obtained from 4 ml of plasma were resuspended in 0·9 ml of Krebs–Ringer bicarbonate buffer from which the calcium and magnesium ions had been omitted.

One-tenth ml of buffer containing debrisoquin-^{14}C (0·1 μc) was added to each suspension of platelets and the samples incubated up to 120 min in a Dubnoff metabolic shaker under 95% oxygen–5% CO_2. After incubation, the platelets were sedimented at 22,000 *g* for 5 min, the supernatants decanted and the tubes swabbed with a cotton-tipped applicator. The samples were weighed and then lysed in 1 ml of distilled water. Aliquots of each supernatant and lysate (0·5 ml) were added to 10 ml of scintillation medium and radioactivity determined as previously described.[5] In all measurements of radioactivity, each sample was counted for sufficient time to accumulate 10,000 counts. The radioactivity of all samples exceeded eight times background. Counting efficiency of individual samples, determined by the channels ratio method

was 72–76 per cent. The distribution ratio of debrisoquin-^{14}C ([I]/[O]) was expressed as the ratio of dpm per milliliter of intracellular platelet water to dpm per milliliter of incubation medium. Total platelet water was 76% and extracellular water 27% of the wet weight of the platelet pellet.[6]

Debrisoquin-^{14}C (S.A. 47·7 μc/mg) was prepared by Dr. H. Kaegi of Hoffmann-La Roche, Inc., Nutley, N. J. In preliminary experiments, radioactivity in platelet lysates was analyzed by thin-layer chromatography on silica gel utilizing a solvent system composed of 95% ethanol:6N HCl (85:15). Greater than 93 per cent of the radioactivity migrated with authentic debrisoquin-^{14}C (R_f 0·73).

The effect of various metabolic inhibitors was studied on the uptake of debrisoquin-^{14}C by the platelet. These include sodium fluoride, 2,4-dinitrophenol (DNP), iodoacetic acid, sodium cyanide and *p*-chloromercuribenzoic acid (PCMB). The initial concentration of these compounds in the incubation medium ranged from 10^{-4} M to 10^{-3} M.

The effect of replacing sodium ion by lithium on the uptake of debrisoquin-^{14}C was also assessed. Platelets were washed twice in isotonic lithium chloride containing 1·3 mM EDTA and incubated in a modified Krebs–Ringer bicarbonate buffer which contained lithium chloride (120 mM) rather than sodium chloride.

The kinetics of uptake of debrisoquin-^{14}C by the platelet were evaluated by relating the initial velocity of uptake of the drug to its concentration in the medium according to the technique of Lineweaver and Burk.[7] The effect of various drugs on the initial velocity of uptake of debrisoquin-^{14}C was also characterized. These included desmethylimipramine (DMI), serotonin (5-HT), tyramine, guanethidine, bretylium tosylate, reserpine phosphate and ouabain.

RESULTS

Human platelets concentrated debrisoquin-^{14}C against a gradient; the steady state distribution ratio ([I]/[O]) reached at 90 min of incubation was 37 at an initial debrisoquin-^{14}C concentration of $5{\cdot}8 \times 10^{-6}$ M. Uptake of the drug was markedly reduced in the cold (Fig. 1).

At an extracellular concentration of $5{\cdot}8 \times 10^{-6}$ M, the distribution ratio of debrisoquin-^{14}C was 21·7 after 60 min of incubation. Increasing the extracellular concentration of drug, decreased the distribution ratio (Fig. 2); at an extracellular concentration of $9{\cdot}6 \times 10^{-5}$ M, the distribution ratio was 7·2. When platelets previously incubated in buffer containing debrisoquin-^{14}C ($5{\cdot}8 \times 10^{-5}$ M) for 1 hr were re-incubated in debrisoquin-free buffer, 57·2 $\pm$ 6·3 per cent (mean $\pm$ S.E.) of the radioactivity was lost from the cells after 1 hr. Serotonin ($2{\cdot}5 \times 10^{-5}$ M) and reserpine (1×10^{-6} M) did not alter this rate of loss.

The effect of various metabolic inhibitors on the uptake of debrisoquin-^{14}C by the platelet is shown in Table 1. The steady-state distribution of debrisoquin-^{14}C was reduced by PCMB, sodium fluoride, iodoacetic acid, sodium cyanide and dinitrophenol.

The substitution of lithium ions for sodium in the preincubation wash and the incubation medium, produced a 39·6 $\pm$ 6·2 per cent (mean $\pm$ S.E.) inhibition of the uptake of debrisoquin-^{14}C by the platelet.

A linear relationship was demonstrated between the reciprocals of the initial velocity of uptake of debrisoquin-^{14}C by the platelet and the concentration of the

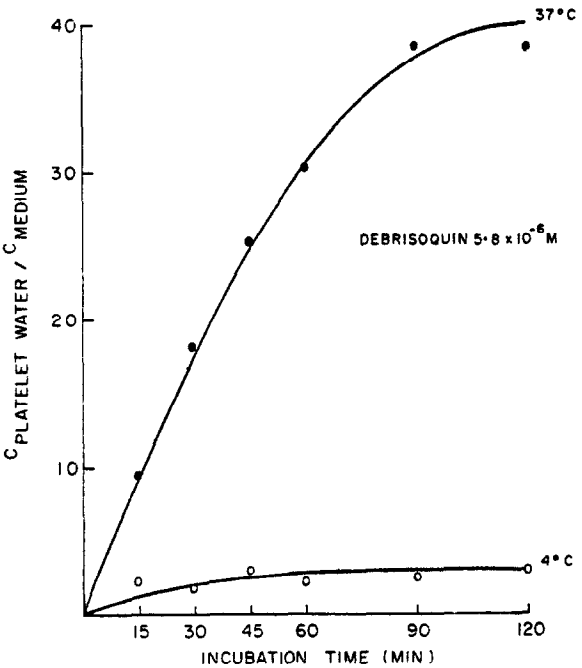


FIG. 1. Rate of accumulation of debrisoquin-^{14}C by the platelet. Washed platelets were incubated with debrisoquin-^{14}C in modified Krebs–Ringer bicarbonate buffer, pH 7·4, at 37 and 4°. Results of representative experiments are shown.

TABLE 1. EFFECT OF METABOLIC INHIBITORS ON THE UPTAKE OF DEBRISOQUIN-^{14}C BY THE PLATELETS*

Inhibitor	Concentration in medium	Per cent inhibition of uptake (Mean ± S.E.)
Iodoacetic acid	1×10^{-3} M	22·2 ± 5·5 (4)†
Sodium fluoride	1×10^{-3} M	24·5 ± 5·9 (6)
Sodium cyanide	1×10^{-3} M	34·3 ± 10·0 (3)
DNP	1×10^{-4} M	19·3 ± 4·2 (6)
PCMB	1×10^{-4} M	38·2 ± 10·0 (5)

* Washed platelets were incubated with debrisoquin-^{14}C ($5{\cdot}8 \times 10^{-6}$ M) and various inhibitors in a modified Krebs–Ringer bicarbonate buffer for 60 min at 37°.

† The figures in parentheses refer to the number of experiments.

drug in the medium (Fig. 3). The K_m of debrisoquin was $4{\cdot}4 \times 10^{-5}$ M and V_{max} $7{\cdot}17 \times 10^{-4}$ M/l. platelet water per hour.

In the presence of desmethylimipramine (10^{-6} M) the velocity of uptake of debrisoquin-^{14}C was reduced (Fig. 3). The common ordinate intercept indicates that desmethylimipramine is a competitive inhibitor of the uptake of debrisoquin-^{14}C by the platelet. Other drugs such as serotonin, tyramine, bretylium and guanethidine also competitively inhibited the uptake of debrisoquin-^{14}C (Fig. 4). Bretylium and

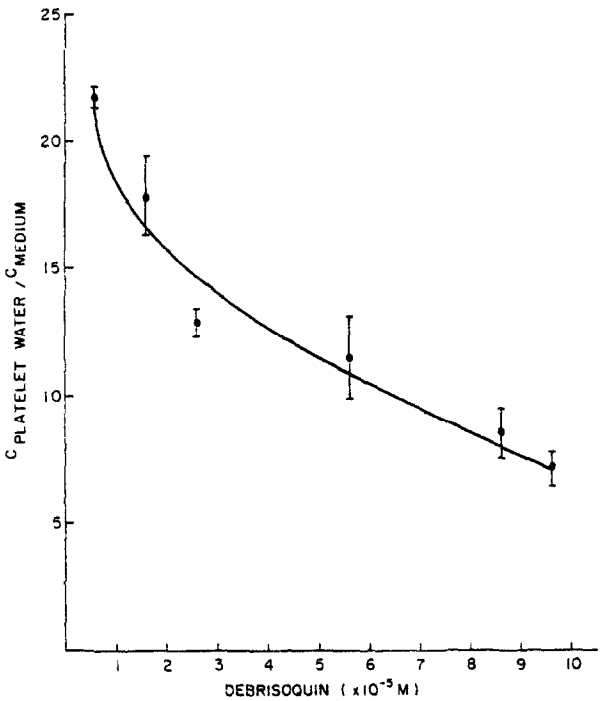
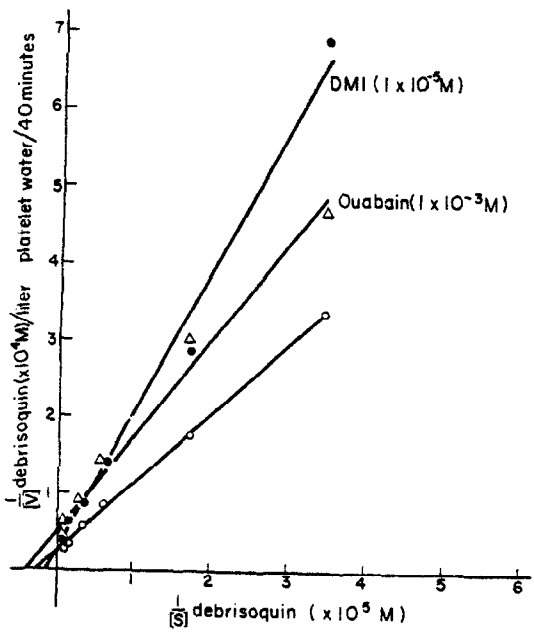


FIG. 2. The effect of concentration of substrate on the distribution ratio of debrisoquin-^{14}C. Washed platelets were incubated with debrisoquin-^{14}C in modified Krebs–Ringer bicarbonate buffer, pH 7·4, for 60 min at 37°. Mean ± S.E. of four experiments are shown.



G. 3. The initial velocity of uptake of debrisoquin-^{14}C by human platelets and the effect of DMI ouabain. Each point represents the mean of 4 control debrisoquin-^{14}C uptake studies and those he inhibitors, a typical experiment. The lines were fitted by linear regression analysis, and model for the data is appropriate at the 0·01 level of significance.

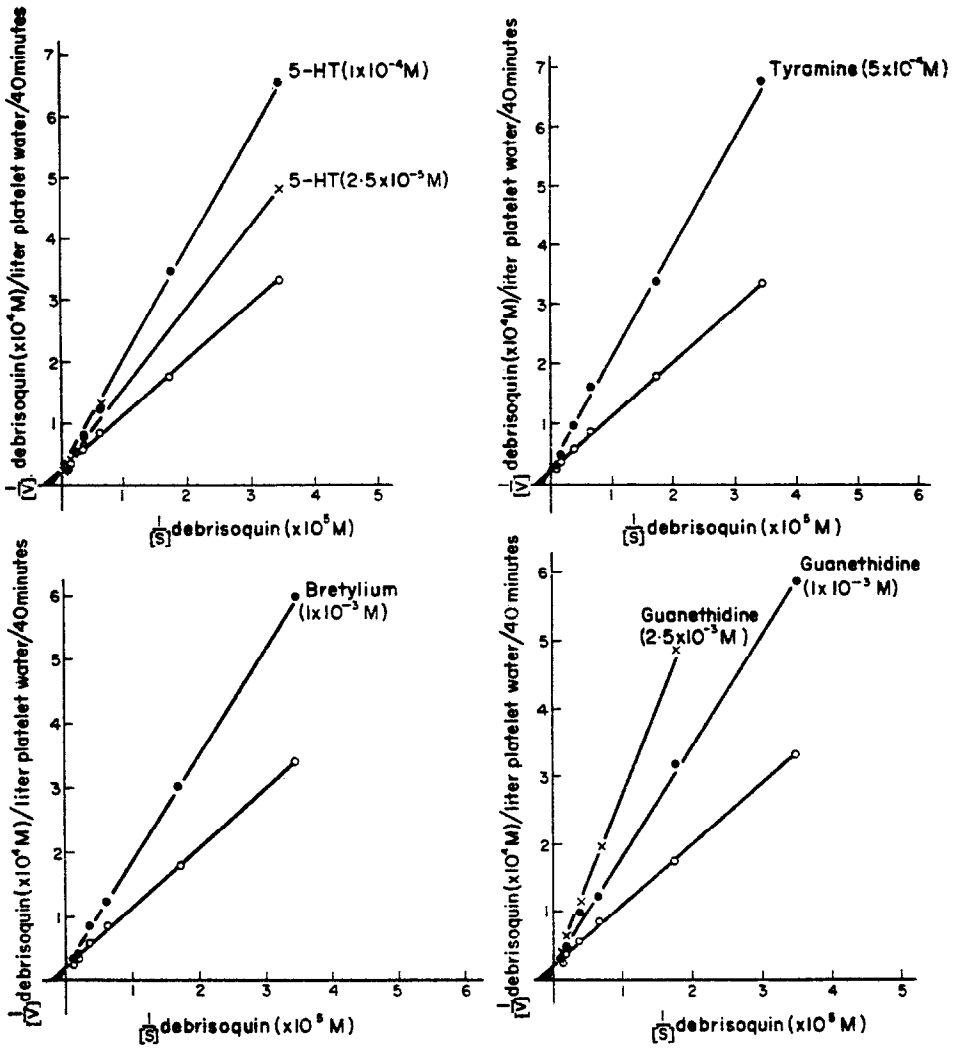


FIG. 4. The effect of various drugs on the initial velocity of the uptake of debrisoquin-^{14}C by human platelets.

TABLE 2. ESTIMATION OF K_i FOR VARIOUS DRUGS INHIBITING THE UPTAKE OF DEBRISOQUIN-^{14}C BY THE PLATELET

Drug	Concentration in medium	K_i
DMI	1×10^{-6} M	$9{\cdot}2 \times 10^{-6}$ M
Serotonin	1×10^{-4} M	$9{\cdot}2 \times 10^{-5}$ M
	$2{\cdot}5 \times 10^{-5}$ M	$4{\cdot}7 \times 10^{-5}$ M
Tyramine	5×10^{-4} M	$5{\cdot}5 \times 10^{-4}$ M
Bretylium	1×10^{-3} M	$1{\cdot}2 \times 10^{-3}$ M
Guanethidine	$2{\cdot}5 \times 10^{-3}$ M	$1{\cdot}1 \times 10^{-3}$ M
	1×10^{-3} M	$1{\cdot}2 \times 10^{-3}$ M

guanethidine were relatively weak inhibitors but desmethylimipramine was very potent (Table 2).

In the presence of ouabain (1×10^{-3} M) the velocity of uptake of debrisoquin-^{14}C was reduced (Fig. 3). Ouabain, however, was not a competitive inhibitor. Reserpine (1×10^{-6} M) did not effect the velocity of uptake.

DISCUSSION

In recent years there has been considerable interest in the uptake and storage of various biogenic amines by the adrenergic neuron. The human platelet, a cell which can be more readily isolated than the adrenergic neuron, actively accumulates amines such as serotonin[8] and norepinephrine.[9] Preliminary studies indicate that the platelet also concentrated the adrenergic blocking agents, guanethidine[10] and debrisoquin.[11]

The present study demonstrates that the human platelet accumulates debrisoquin against a concentration gradient. Such accumulation was reduced in the cold and by cyanide and DNP, inhibitors of aerobic metabolism, as well as by fluoride, an inhibitor of anaerobic metabolism. These inhibitors also reduce the active transport of norepinephrine,[9] as well as amino acids such as glycine and *alpha* aminoisobutyric acid,[12] into this cell. Uptake of debrisoquin-^{14}C by the platelet is energy dependent and saturable, which suggests that the drug is actively transported by the cell.

PCMB, an agent which markedly alters the character of the platelet membrane,[13] diminished the uptake of debrisoquin-^{14}C. These results suggest that the active transport process for this compound is located within the membrane.

The K_m for the active uptake of debrisoquin-^{14}C was $4{\cdot}4 \times 10^{-5}$ M. Serotonin, an amine which is actively transported by the platelet, was a competitive inhibitor of the uptake of debrisoquin-^{14}C ($K_i = 4{\cdot}7 \times 10^{-5}$ M). In previous studies[3] of the uptake of serotonin by the platelet, the K_m for the transport of this compound was $4{\cdot}5 \times 10^{-5}$ M and the K_i of debrisoquin was $4{\cdot}4 \times 10^{-5}$ M. Thus the K_i of debrisoquin against the uptake of serotonin was the same as the K_m of debrisoquin-^{14}C for the uptake process. Similarly, the K_i of serotonin against the uptake of debrisoquin-^{14}C was the same as the K_m of serotonin. These results suggest that debrisoquin and serotonin share a transport process in the platelet, and have an equal affinity for the process. This active transport mechanism in the platelet for organic bases resembles the membrane amine pump of the adrenergic neuron; both are relatively nonspecific.

DMI, a potent inhibitor of the uptake of norepinephrine by the platelet[9] and the adrenergic neuron,[14] markedly reduced the uptake of debrisoquin-^{14}C by the platelet. It has been demonstrated that DMI competitively inhibits the uptake of l-metaraminol into rabbit myocardial slices.[15] Similarly, this drug was a competitive inhibitor of the uptake of serotonin[16] and debrisoquin-^{14}C by the platelet.

The maximal accumulation of debrisoquin by the platelet was dependent upon the concentration of sodium ions in the incubation medium. Uptake of the drug was reduced when lithium ions were substituted for sodium and when ouabain was added to the incubation medium. Similar effects of lithium and ouabain on the uptake of norepinephrine by the human platelet[9] and adrenergic neuron[17, 18] have been noted. It has been reported that the uptake of l-metaraminol by heart slices was noncompetitively inhibited by ouabain.[15] In the present study ouabain altered both the V_{max} of the uptake of debrisoquin-^{14}C by the platelet as well as the K_m. These results are compatible with a mixed type of inhibition.

When platelets incubated in a medium containing debrisoquin-^{14}C were resuspended in drug-free medium, radioactive drug was rapidly lost from the cells. Recent studies have demonstrated that the rate of release of debrisoquin from rabbit myocardial slices is also rapid.[19] In contrast, the depletion of serotonin from platelets treated in a similar manner occurs quite slowly.[20]

Moreover reserpine, a potent releaser of serotonin from storage granules within the platelet,[21] does not effect the accumulation or rate of loss of debrisoquin from this cell or from myocardium.[19] These results suggest that debrisoquin and serotonin do not have the same intracellular distribution. It is possible that intracellular binding site(s) for organic bases in the platelet have greater specificity than the active transport process for these compounds.

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